

**[0016]** The terms “nucleic acid,” “polynucleotide,” and “oligonucleotide” include a polymeric form of nucleotides of any length, including, but not limited to, ribonucleotides or deoxyribonucleotides. There is no intended distinction in length between these terms. Further, these terms refer only to the primary structure of the molecule. Thus, in certain embodiments these terms can include triple-, double- and single-stranded DNA, as well as triple-, double- and single-stranded RNA. They also include modifications, such as by methylation and/or by capping, and unmodified forms of the polynucleotide. More particularly, the terms “nucleic acid,” “polynucleotide,” and “oligonucleotide,” include polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing nonnucleotidic backbones, for example, polyamide (e.g., peptide nucleic acids (PNAs)) and polymorpholino (commercially available from the Anti-Virals, Inc., Corvallis, Oreg., as Neu-gene) polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

**[0017]** A “primer” is a single-stranded polynucleotide capable of acting as a point of initiation of template-directed DNA or RNA synthesis under appropriate conditions (i.e., in the presence of four different nucleoside triphosphates and an agent for polymerization, such as, DNA or RNA polymerase or reverse transcriptase) in an appropriate buffer and at a suitable temperature. The appropriate length of a primer depends on the intended use of the primer but typically is at least 7 nucleotides long and, more typically range from 10 to 30 nucleotides in length. Other primers can be somewhat longer such as 30 to 50 nucleotides long. In this context, primer “length” refers to the portion of an oligo- or polynucleotide that hybridizes to a complementary “target” sequence and primes synthesis. For example, in the primer 5'-U<sub>1</sub>-B<sub>1</sub>-F<sub>1</sub>-3' the length of F<sub>1</sub> might be 20 nucleotides and the combined length of U, B and F<sub>1</sub> could be 60 nucleotides or more (typically between 30 and 100 nucleotides). Short primer molecules generally require cooler temperatures to form sufficiently stable hybrid complexes with the template. A primer need not reflect the exact sequence of the template but must be sufficiently complementary to hybridize with a template. The term “primer site” or “primer binding site” refers to the segment of the target DNA to which a primer hybridizes. The term “primer pair” means a set of primers including a 5' “upstream primer” that hybridizes with the complement of the 5' end of the DNA sequence to be amplified and a 3' “downstream primer” that hybridizes with the 3' end of the sequence to be amplified.

**[0018]** A primer or probe anneals or hybridizes to a complementary target sequence. The primer or probe may be exactly complementary to the target sequence or can be less than perfectly complementary. Typically the primer has at least 65% identity to the complement of the target sequence over a region of at least 7 nucleotides, more typically over a region in the range of 10-30 nucleotides, and often over a region of at least 14-25 nucleotides, and more often has at least 75% identity, at least 85% identity or 90% identity. It will be understood that certain bases (e.g., the 3' base of a primer) generally should be exactly complementary to corresponding base of the target sequence. Primer and probes generally anneal to the target sequence under stringent conditions.

Stringent annealing conditions refers to conditions in a range from about 5° C. to about 20° C. or 25° C. below the melting temperature ( $T_m$ ) of the target sequence and a probe with exact or nearly exact complementarity to the target. As used herein, the melting temperature is the temperature at which a population of double-stranded nucleic acid molecules becomes half-dissociated into single strands. Methods for calculating the  $T_m$  of nucleic acids are well known in the art (see, e.g., Berger and Kimmel (1987) *METHODS IN ENZYMOLOGY*, VOL. 152: *GUIDE TO MOLECULAR CLONING TECHNIQUES*, San Diego: Academic Press, Inc. and Sambrook et al. (1989) *MOLECULAR CLONING: A LABORATORY MANUAL*, 2ND ED., VOLS. 1-3, Cold Spring Harbor Laboratory), both incorporated herein by reference). As indicated by standard references, a simple estimate of the  $T_m$  value may be calculated by the equation:  $T_m = 81.5 + 0.41(\% \text{ G+C})$ , when a nucleic acid is in aqueous solution at 1 M NaCl (see e.g., Anderson and Young, *Quantitative Filter Hybridization in NUCLEIC ACID HYBRIDIZATION* (1985)). The melting temperature of a hybrid (and thus the conditions for stringent hybridization) is affected by various factors such as the length and nature (DNA, RNA, base composition) of the probe and nature of the target (DNA, RNA, base composition, present in solution or immobilized, and the like), and the concentration of salts and other components (e.g., the presence or absence of formamide, dextran sulfate, polyethylene glycol). The effects of these factors are well known and are discussed in standard references in the art

**[0019]** A “probe” is an nucleic acid capable of binding to a target nucleic acid of complementary sequence through one or more types of chemical bonds, usually through complementary base pairing, usually through hydrogen bond formation, thus forming a duplex structure. The probe binds or hybridizes to a “probe binding site.” The probe can be labeled with a detectable label to permit facile detection of the probe, particularly once the probe has hybridized to its complementary target. The label attached to the probe can include any of a variety of different labels known in the art that can be detected by chemical or physical means, for example. Suitable labels that can be attached to probes include, but are not limited to, radioisotopes, fluorophores, chromophores, mass labels, electron dense particles, magnetic particles, spin labels, molecules that emit chemiluminescence, electrochemically active molecules, enzymes, cofactors, and enzyme substrates. Probes can vary significantly in size. Some probes are relatively short. Generally, probes are at least 7 to 15 nucleotides in length. Other probes are at least 20, 30 or 40 nucleotides long. Still other probes are somewhat longer, being at least 50, 60, 70, 80, 90 nucleotides long. Yet other probes are longer still, and are at least 100, 150, 200 or more nucleotides long. Probes can be of any specific length that falls within the foregoing ranges as well.

**[0020]** The term “label” refers to a molecule or an aspect of a molecule that can be detected by physical, chemical, electromagnetic and other related analytical techniques. Examples of detectable labels that can be utilized include, but are not limited to, radioisotopes, fluorophores, chromophores, mass labels, electron dense particles, magnetic particles, spin labels, molecules that emit chemiluminescence, electrochemically active molecules, enzymes, cofactors, enzymes linked to nucleic acid probes and enzyme substrates. The term “detectably labeled” means that an agent has been conjugated with a label or that an agent has some inher-